PB-216 759

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6 September 1950

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NATIONAL TECHNICAL
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SENSITIZING THE 4-AMINOANTIPYRINE REACTION WITH PHENOLIC MATERIALS FOR USE IN WATER AND WASTE ANALYSIS*

The use of 4-aminoantipyrine was proposed by Emerson [1] in a new color test for phenols. As outlined by Emerson, the essentials of the reaction consist of bringing together the phenolic material, the 4-aminoantipyrine and an alkaline oxidant in a solution of sufficiently high pH to prevent the formation of antipyrine red. Potassium ferricyanide was recommended as the oxidant. Gottlieb and Marsh [4] developed a procedure for the application of Emerson's reagent (4-aminoantipyrine) to the determination of certain phenolic fungicides. Martin [5] described a procedure for the "Rapid Colorimetric Estimation of Phenol" which consists essentially of a slight modification of the Gottlieb and Marsh procedure.

According to Emerson and Berglas [2] many ring compounds give the aminoantipyrine test. All the compounds which give a positive test have an enol-keto system incorporated into a ring structure, but not all such systems give a positive test. Emerson [1] carefully outlined the types of phenolic materials which should respond to the test. In their work, Gottlieb and Marsh noted two phenolic materials which apparently do not conform to Emerson's rules.

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* Presented at the 118th National Meeting of the American Chemical Society, Chicago, Illinois, September 3-8, 1950

All investigators cited have used potassium ferricyanide as the alkaline oxidant in the application of the reaction to the detection of phenolic materials. In the matter of pH control, Emerson (1) made no definite recommendations except that the pH be high enough to prevent the formation of antipyrine red. Gottlieb and Marsh (3) recommended pH values of 10.4 to 10.6 produced with solutions of sodium carbonate without citing any data which demonstrated that the particular range specified was definitely required or particularly desirable.

Martin (5) tacitly accepted the pH range suggested by Gottlieb and Marsh (4) but used ammonia to produce the desired pH. In addition, Martin applied his procedure to brine solutions and added a definite amount of ammonia which leaves considerable doubt as to the pH which actually may have been produced in some of his reaction mixtures.

On the basis of the work reported, the use of Emerson's reaction promises the possibility of many advantages. These apparently include: speedy results, ease of manipulation, the use of relatively stable reagents and applicability over a wide range of concentrations of phenolic material. However, better evaluation of the variables involved in the reaction and, if possible, great increase in reaction sensitivity, are needed before this promising test attains its full usefulness.

The work to be described has had two goals:

(1) Evaluation of the reaction variables which affect usage of Emerson's reagent for the determination of the simpler phenols.

(2) Devising a procedure sufficiently sensitive to detect a one p.p.b. concentration of phenol.

In order to increase the sensitivity of the reaction, it is obvious that concentration of the dye is required. Using the procedure described by Martin [5] to produce the dye, a number of solvents were tried. In general, it was found that chlorinated hydrocarbons such as chloroform, ethylene dichloride, carbon tetrachloride and tetrachloroethane could be used to extract the dye from the reaction mixture. Chloroform was selected for further study for a number of reasons:

(1) Its use as an extractant had been previously investigated in some detail by Emerson [1].

(2) It appeared to be the best of the chlorinated hydrocarbons tried from the standpoint of extraction efficiency, intensity of color of the extract, stability of extracted color, cost, availability and toxicity.

The notable results of the extraction with chloroform were:

(1) The red aqueous colors of the commoner phenols with absorption maxima at approximately 510 millimicrons were extracted as yellow or orange solutions having an absorption maxima in the neighborhood of 460 millimicrons.

(2) The potassium ferricyanide which gave the reaction mixture a yellow color was not extracted in any appreciable amount.

(3) A slight yellow color showed up in blanks, apparently due to the reaction of the reagent (4-aminoantipyrine) with the oxidant.

On the basis of qualitative observations, it was judged that the chloroform extracts were more stable than the aqueous reaction mixture and it was deemed advisable to base the investigation of the reaction on chloroform extracts rather than aqueous reaction mixtures. In undertaking this step, it was recognized that pH differences might result in variable extraction efficiency. Subsequently this possible difficulty failed to materialize. On the basis of visual observation of serial extractions, the extraction procedures used apparently gave substantially quantitative extraction in all the work reported in this paper.

Effect of pH

In undertaking to evaluate the several variables which might be entailed in this reaction, the effect of pH was investigated first. The procedure outlined below was used:

Reagents

Ammonium hydroxide, concentrated, C.P.

Potassium ferricyanide solution - A 2.0 per cent solution of the C.P. salt in distilled water.

4-aminoantipyrine solution - A 3.0 per cent aqueous solution of 4-aminoantipyrine*.

Apparatus Used

Beckman Model B spectrophotometer.

1 cm. and 5 cm. absorption cells (Corex).

Beckman Model H-2, pH meter equipped with blue point, high pH Beckman electrode.

Procedure

To a 300 ml. portion of sample add sufficient concentrated ammonia to produce the pH desired in the final reaction mixture. (This may require some cut and try work). Mix well. Add 2 ml. of aminoantipyrine solution and mix again. Add 20 ml. of the potassium ferricyanide solution and mix again. Start the extractions with chloroform after three minutes. Use 25 ml. for the first extraction

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(*This chemical may be obtained from the Special Chemical Division of Winthrop-Stearns, New York 13, New York).

and 15 ml. for each of two successive extractions. Combine the three extracts. Dilute the combined extract up to 50 ml. with chloroform and compare with a corresponding reagent blank.

The results of some of this work applied to phenol are shown in Figure 1. Figure 2 shows the results of a similar study carried out with o-cresol. Moreover, in Figure 2, both the readings obtained with the blank and with the sample, against chloroform are shown. It will be noted that there is a relatively constant differential between the blank and the chloroform over a wide pH range. This constant differential is the result of a similar decrease in the reading of both the sample and the blank. When the use of this reaction is attempted for either high sensitivity or high precision, this variation will be one of the limiting factors. For extreme sensitivity or accuracy, it would obviously be necessary to control the pH so that values as closely identical as possible are obtained on all samples and standards.

Aniline is one of the more common materials among the many compounds described by Emerson as reacting with the aminoantipyrine reagent. There is some possibility, however, that it may be representative of a fair share of the materials which may be expected to give positive interference with the use of Emerson's reagent as a test for phenols. For this reason, solutions containing 100 p.p.m. of aniline were put through the same pH effect study as was just

described. The results of this work are shown in Figure 3. This work indicates the desirability of carrying out the phenol test at the maximum feasible pH. However, at pH values of 9.8 or above, 20 p.p.m. of aniline are required to give as much color as 0.1 p.p.m. of phenol.

Study of the results indicated that the pH range from 9.8 to 10.2 would be suitable in most cases for use with a system in which the pH is controlled by ammonia. At pH values above 10.2 small differences in pH apparently produce considerable differences in the intensity of the color. The use of lower pH values appears to be undesirable because aniline and probably other undesired keto-enol systems which might be present show a greater tendency to respond to the test. The choice is not really clear cut when trace quantities of phenol are sought, but the range given appears to be about as good as can be selected. For high precision work, or work involving high sensitivity, it appears desirable but impractical to suggest a more narrow range.

4-Aminoantipyrine Requirements

The amount of 4-aminoantipyrine required to most advantageously carry out the reaction was next investigated. For the initial attack on this problem, a study was made of the effect of the amount of 4-aminoantipyrine on the color produced by an otherwise constant system. Three hundred ml. of a distilled water solution containing 100 p.p.b. of phenol were used as test portions. In all cases

sufficient ammonia was used to produce a final pH in the range previously selected (9.8-10.2). The amount of ferricyanide was held constant at the addition previously used. The results of this study are presented graphically in Figure 4.

Figure 4 demonstrates two things:

(1) The amount of color of the blank appears to be proportional to the amount of 4-aminoantipyrine used.

(2) In spite of the speed of the reaction, relatively huge excesses of 4-aminoantipyrine are required to reproduce the maximum color.

Careful consideration indicated that it was desirable to standardize on 2.0 ml. of 3.0 per cent aminoantipyrine solution for 300 ml. samples. This amount of the chemical produced maximum color and provided a safe but not gross excess of the reagent, without giving an excessively dark blank.

As previously stated, one of the primary objects of this work is to develop a procedure having maximum sensitivity. Accordingly, the effect of variation in reagent addition on the color produced in liter portions was investigated. For this work, a solution containing 30 p.p.b. of phenol was used as a test media. The pH was again held in the previously selected range by additions of ammonia. The amount of ferricyanide solution used was held constant at 20 ml. of 2.0 per cent solution. The extraction was carried out as a set of three serial extractions, 25, 20 and 15 ml. portions of chloroform being used and the combined extracts made up to 50 ml. for color comparison. The

results of this work are shown in Figure 5. Figure 5 indicates again that the blank is proportional to the amount of 4-aminoantipyrine reagent used. Comparing the reagent blank curves of Figure 5 with those of Figure 4, it is apparent that both the amount of 4-aminoantipyrine reagent and its concentration have a definite bearing on the amount of color developed by a reagent blank. Figure 5 further indicates that the amount of reagent required for the development of maximal color was not included in the range of the reagent concentrations used. However, the small amount of aminoantipyrine was again selected for further development and evaluation. The curve indicates that slight variations in the amount of reagent added will have relatively small effect on the intensity of the color production. At the same time the color of the blank is kept at a point where the color produced by the phenol present is high in relationship to that of the blank. The use of smaller amounts would introduce too much instability of the reaction due to possible errors in control of reagent additions. The use of larger amounts would result in blanks having excessive color in comparison with the color which would be developed by trace amounts of phenol.

Effect of Temperature

The effect of temperature was studied to determine whether this factor is an important variable. Table 1 shows the results of parallel tests carried out at 30° and 37°C. In this work, all solutions and reagents were brought to 30° and 37°C. and the extractions carried

out inside incubators at the corresponding temperatures. The spectrophotometric measurements were made at room temperature. This created a difficulty. The 37°C. extracts cooled and fogged up due to separation of water from solution. To overcome this difficulty, the 37°C. extracts were centrifuged and again made up to volume with chloroform. This extra manipulation is believed to be in some measure responsible for the relatively small differences shown between the 20° and 37 C. results. The 37°C. blank control showed 40 per cent more color than the 20° control. Here again the differential color was relatively independent of the variable, while the blank was not.

Temperature is obviously a factor which would be involved in the rates of fading and color change of either aqueous reaction mixtures or chloroform extracts. However, as will be shown later, proper celerity of manipulation should control this variable effectively.

In any work involving attempts to attain either extreme sensitivity or high precision, temperature is also important because of its possible differential effect on the "blank". The proper procedure is to hold all reacted portions at the same temperature so that they are the same.

Order of Addition of Reagents

In carrying out the reaction between the *p*-aminoacetophenone and phenols, the order of addition of reagents may be important. The pH should be adjusted before the *p*-aminoacetophenone is introduced. Emerson's observations suggest that many oxidants will react with the aminoacetophenone to produce a violet dye at the pH values which a sample might have. The production of color by *p*-aminoacetophenone with potassium ferricyanide

shows a somewhat unpredictable behavior. Thus, if 4-aminoantipyrine and potassium ferricyanide are added to distilled water, a deep red color develops. If, however, (1) the pH is adjusted to 10 with ammonia, (2) the 4-aminoantipyrine and potassium ferricyanide added and (3) the pH lowered by gradual increments of a dilute acid, substantial amounts of color fail to appear. This is true even when the pH values are reduced well below those produced when the 4-aminoantipyrine-potassium ferricyanide system is added to distilled water. Similar results are obtained if sodium carbonate is substituted for ammonia to raise the pH. This observation is difficult to explain from the theoretical standpoint. The obvious practical implication is that pH adjustment should precede the introduction of the 4-aminoantipyrine to minimize blank reactions in case an oxidant, or some material which catalyzes oxidation by dissolved oxygen, is present.

It may be noted that the Gottlieb and Marsh [4] procedure introduces the alkali before the 4-aminoantipyrine. Martin's procedure, however, calls for addition of the 4-aminoantipyrine first, thus introducing the 4-aminoantipyrine into a system of uncontrolled pH. In most cases, the order of addition of the alkali and the aminoantipyrine probably would make little difference, however, in certain cases it might be important. For this reason, it is desirable to routinely add the alkali first.

Effect of Solution Age on Color

In the preceding work, it has been assumed that short periods of storage, 10 minutes or less, had little effect on the optical densities of the aqueous reaction mixtures and that periods of storage of an hour made little difference in the color of the chloroform extracts. When any variable was under study, a corresponding reagent blank and sample were prepared simultaneously. Actually the assumption has been that the color differential of solutions and blanks was relatively constant for a period of five or ten minutes.

To evaluate the effect of storage, observations were made on the color of stored blanks and samples. Figure 6 shows the results of such a study of aqueous reaction mixtures used in the 300 ml. chloroform extraction procedure. This figure demonstrates that:

(1) the blank darkens progressively,

(2) the sample fades at first and then starts to darken as the darkening of the blank exceeds the fading of the dye produced,

(3) the dye produced by the reaction undergoes a continuous slow fading.

In terms of measurements based on observations on aqueous reaction mixtures, the results indicate the need for speedy manipulation and color comparison. In terms of measurements based on chloroform extractions, the starting of the extraction immediately after mixing of the reagents is indicated.

Tables 2A and 2B show the results of studies of the storage of chloroform extracts from phenol reaction mixtures. Table 2A lists absorbancy readings on extracts as separated. Table 2B lists information obtained with the reaction and extraction carried out at 20°C. and the extract filtered to remove the small amounts of water collected along with the chloroform. In general the results indicate satisfactory stability over three-hour periods. The dried extracts in general showed less variation than the wet extracts presumably because there was less light absorption or other effects caused by the small amounts of excess water in the extract. On long holding the absorbancy of the extracts tends to become highly erratic. It might be mentioned that many results similar to those in Table 2B have been obtained using phenol and other simple hydrocarbon substituted phenolic materials.

Effect of Ferricyanide Concentration

The effect of the ferricyanide concentration apparently is not critical in so far as the reaction is concerned. This may be deduced from the similarity of behavior of the 1000 ml. and 300 ml. procedures in which the same amount of ferricyanide was used. With regard to procedures in which the dye is extracted, the extent of the ferricyanide excess is relatively immaterial as this reagent is not extracted. However, when observations are made on aqueous solutions, either visually or with a filter photometer, the control of the reagent may become important, because of its color. Mechanically, this may be in large measure taken care of by selecting the proper wave length for the

observation if a spectrophotometer is available. With either visual or filter photometer observation, variations in ferricyanide concentration, either through loose measurement or reaction, may become consequential. This makes it necessary to prevent occurrence of substantial ferricyanide differentials through careful measurement and by removal of non-phenolic materials which react with this reagent.

Absorption Characteristics of Reaction Products

The light absorption characteristics of the materials produced by the interaction of 4-aminoantipyrine and some of the simpler phenols are presented in Tables 3A and 3B. Table 3A presents the results obtained comparing aqueous solutions with an aqueous blank; Table 3B shows similar data on chloroform extracts compared to corresponding blanks. It is of interest to note, that most of the aqueous curves have maxima in the vicinity of 510 millimicrons and most of the chloroform extracts show maxima near 460 millimicrons, the wave lengths used for quantitative comparison. However, very different absorption characteristics are shown by some phenolic materials at other wave lengths.

On the basis of comparisons of results with aqueous solutions at 510 millimicrons, none of the phenolic materials so far investigated produce more absorbancy per unit weight of material than does phenol itself. Regarding chloroform extracts, a similar statement is true for absorbancy measurements at 450-460 millimicrons. Further, most of the phenolic materials studied have absorbancy maxima at or near these

wave lengths. This characteristic of the reaction permits a definite interpretation of the analysis of solutions containing mixed phenols. When reported as phenol, results obtained by spectrophotometric measurements at the wave lengths enumerated represent the minimum amount of phenolic materials which may be present.

The sensitivity of the test varies with different phenols. Table 4 lists some of the characteristics of the reaction products and indicates the approximate threshold of positive observation. These data show the wide variation in the reaction of some phenols to the test. Those simple phenols which gave a red color showed a linear response to the test.

Standards and Precision

In view of the variable nature of the absorption curves produced by different phenols, the use of visual comparisons for the estimations of phenol in the examination of samples containing mixed phenols is highly undesirable. Such practice is likely to produce results which cannot serve as the basis for any definite interpretation.

For routine work, it is possible to establish standard absorbancy equations or curves, or transmission curves. For high precision, however, it is desirable to run a new standard each time. Such practice will correct for variations in reagent quality.

The filtration of chloroform extracts is an essential procedure when either maximal sensitivity or extreme precision is desired. This procedure removes emulsified water and apparently tends to make chloroform extracts slightly more stable.

Procedures Recommended

The procedures listed previously have been set up primarily for use as tools for the study of the variables involved in the reaction under investigation. Although any one of them is serviceable within the limitations indicated, they do not necessarily represent the most convenient method of analysis for water or waste samples. Furthermore, the essentially loose nature of the basic reactions must be considered. Before the reaction is applied, every effort should be made to screen out interfering material. Such materials will include many non-phenolic aromatic compounds having a keto-enol system, strong oxidants, strong reductants, and materials which will color or give opacity to the solvent in which color comparisons are made. As pH control is important, buffering agents and acids and alkalis should also be removed.

An extensive discussion of the preliminary purification of samples for analysis for mixed phenolic materials has been presented by Ettinger and Kroner [5]. This discussion will not be recapitulated here. However, it must be emphasized that successful analysis of many samples will be contingent upon adequate preliminary work before the reaction is applied.

For comparison of aqueous solutions, the 300 ml. procedure previously described with the pH adjusted to 9.8 to 10.2 is considered the best of those studied although it will be found convenient to put the reaction on a 100 ml. basis.

For sensitivity and precision at low concentrations the 1000 ml. procedure previously described is the best. This procedure may be revamped using a 500 ml. sample with half quantities of all reagents without modifying the properties shown by the extract. This reduced volume procedure, designed primarily for the examination of surface waters, is detailed in the following.

Suggested Procedure for the Examination of Polluted Surface Water Samples for Phenol

1. Reagents

   a. 4-aminoanti pyrine solution - a 3.0 per cent solution in distilled water.

   b. Potassium ferricyanide solution - a 2.0 per cent solution of the C.P. chemical dissolved in distilled water and filtered.

   c. Ammonium hydroxide - 6.0 N solution of C.P. ammonium hydroxide.

   d. Chloroform - C.P., A.C.S.

   e. Standard phenol solution - dissolve 1.0 grams of C.P., water white, phenol in 1.0 liter of distilled water.

2. Apparatus

   Beckman Model B spectrophotometer or Beckman Model D.U. spectrophotometer equipped to handle five and ten cm. cuvettes, or other suitable spectrophotometric apparatus.

3. Procedure

a. Purify 500 ml. of the sample suitably, depending upon the interfering impurities in the sample. Adjust the temperatures of the sample or aliquots of the sample used, and blanks and standards described under 3c. so that they are identical.

b. To 500 ml. of sample, or an aliquot diluted to 500 ml., add sufficient 6 N ammonia to produce a pH of 9.8 to 10.2 in the final reaction mixture. Unless materials which interfere with the pH adjustment are present, 1.6 ml. of the 6 N ammonia will produce a pH in the desired range. Check compliance with the specified range after all the reagents are introduced.

c. Simultaneously prepare a 500 ml. blank and 500 ml. standards containing 0.04 p.p.m., 0.20 p.p.m., and 1.0 p.p.m. of phenol by suitable dilution of the standard phenol solution. Adjust the pH so that a reaction mixture having a pH of 10.0 ± 0.2 is obtained. Treat the blanks and controls in parallel with the sample in steps 3 and 3b.

d. Add exactly 1.0 ml. of 3.0 per cent 4-aminoantipyrine to all portions and mix well.

e. Add 10 ml. of 2.0 per cent potassium ferricyanide solution to all portions and mix immediately.

f. Immediately pour 100 ml. of the sample and the 0.2 p.p.m. standard into individual 100 ml. Nessler tubes. If the sample does not show more red color than the standard, consolidate the entire sample portion and put it in a one liter separatory funnel. Also put the blank and the 0.04 p.p.m. standard in one liter separatory funnels. Extract these solutions serially with portions of chloroform of 15 ml., 10 ml., and 5 ml. Combine the extracts and make them up to 25 ml. with chloroform.

g. Read the optical density of both the 0.04 p.p.m. standard and the sample at 460 millimicrons against the blank extract. If the optical density of 5 cm. of liquid is over 1.0, comparisons should be based on readings made using 1 cm. tubes.

h. Compute the phenol content on a proportional basis as the reaction is linear.

i. If at step (d) the sample is darker than the 0.20 standard, start comparisons immediately using the aqueous solutions. Read both the sample and the 1.0 p.p.m. standard against the aqueous blank at 510 millimicrons and calculate the phenol content of the sample on a proportional basis.

Summary

Investigation of the reaction between 4-aminoantipyrine and phenols for analytical purposes indicates the following factors to be significant:

(1) The reaction products may be concentrated by extraction with chloroform in the case of all phenols investigated. The red aqueous dyes are extracted with chloroform to yield orange or yellow solutions. Serial extractions may be so arranged that the extraction is substantially quantitative and a large concentration factor obtained.

(2) The aqueous reaction mixture is unstable. When allowed to stand, the color produced by the reaction of phenol and 4-aminoantipyrine fades. Concurrently, the color due to the reagents increases.

(3) The pH is an important factor in the control of the reaction. The maximum stability of color produced appears to be in the range 9.8 to 10.2. In order to minimize possible interferences, a range of 9.3 to 10.2 is recommended for the reaction mixture.

(4) Temperature is an important factor when small amounts of phenols are sought. Although the reaction appears to be affected only slightly by ordinary temperature variations, such variations affect the blank color significantly.

(5) With the chloroform extraction procedure, the amount of 4-aminoantipyrine used is largely responsible for determining the color of the blank.

(6) The reaction was found to be linear over wide ranges of concentrations for a number of the simpler phenols.

(7) When aqueous solutions are compared to phenol standards at 510 millimicrons or chloroform extracts compared to phenol standards at 460 millimicrons, the results of examination of mixed phenols indicate the minimum amount of phenolic material which may be present.

(8) A procedure is recommended for the examination of surface waters. The recommended procedure is sensitive to about 2 p.p.b. of phenol when a single determination is made. The use of replicate or modified procedures can extend the reliable sensitivity to 1 p.p.b. The background data presented should allow the devising of procedures especially adapted to other uses.

Literature Cited

(1) Emerson, Edgar,
J. Org. Chem., 8, 417 (1943).

(2) Emerson, Edgar, and Beagle, Lindley Clair,
J. Org. Chem., 8, 433 (1943).

(3) Ettinger, M. B., and Kroner, R. C.,
Proc. of the 5th Industrial Waste
Conference, Purdue University (1949).

(4) Gottlieb, S., and Marsh, P. B.,
Ind. and Eng. Chem., Anal. Ed. 18, 16 (1946).

(5) Martin, R. W.,
Anal. Chem., 21, 1419 (1949).

FIGURE 1

EFFECT OF pH ON COLOR FORMATION WITH PHENOL (CHLOROFORM EXTRACTS) (300 ml PROCEDURE, VARYING AMOUNTS OF PHENOL AT VARIOUS TIMES)

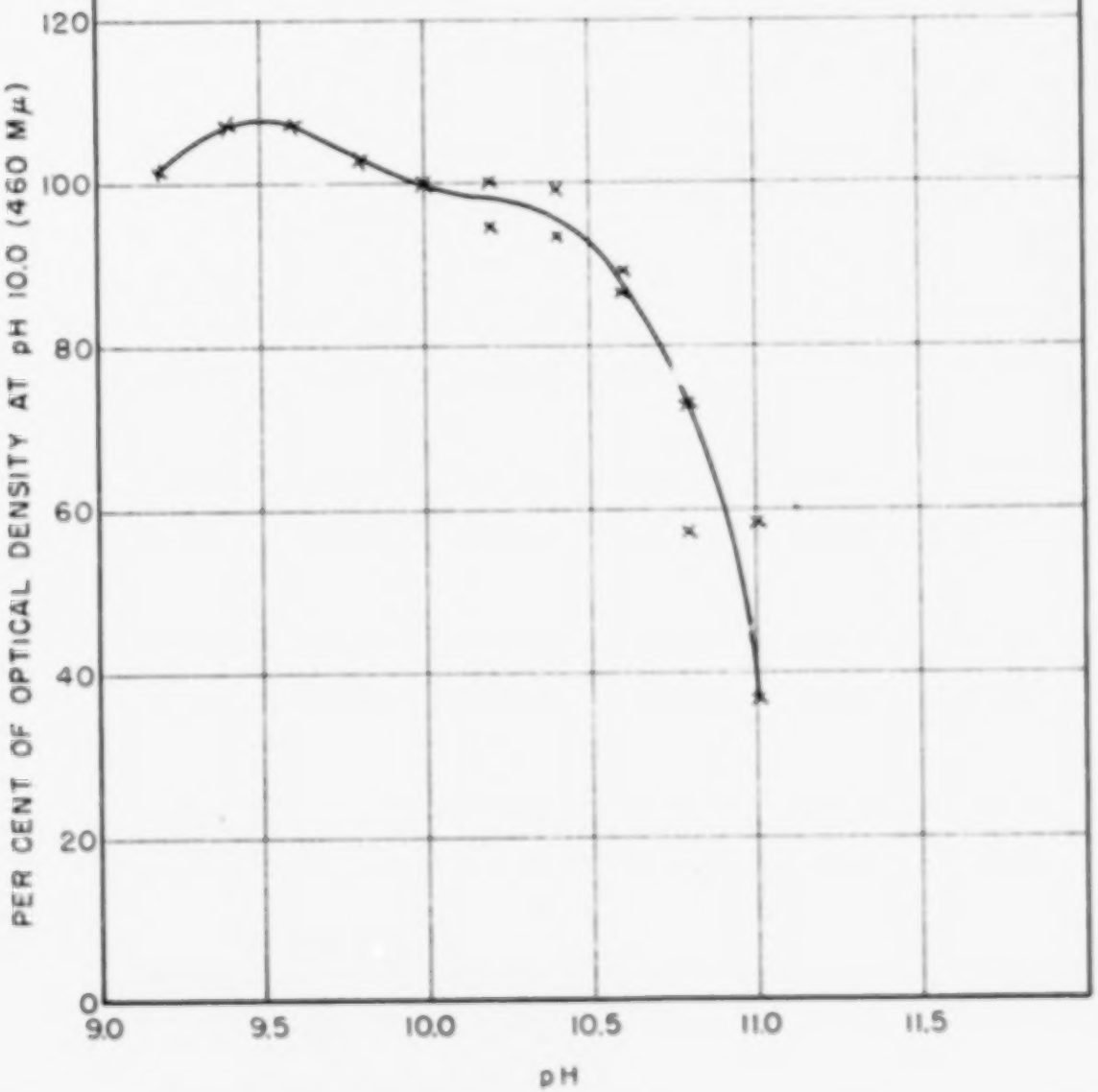

FIGURE 2

Effect of pH on Color Produced from o-Cresol (100 ppb)

300 ml procedure, $CHCl_3$ Extraction Procedure

1. Blank vs chloroform
2. Sample vs chloroform
3. Differential-color due to o-cresol

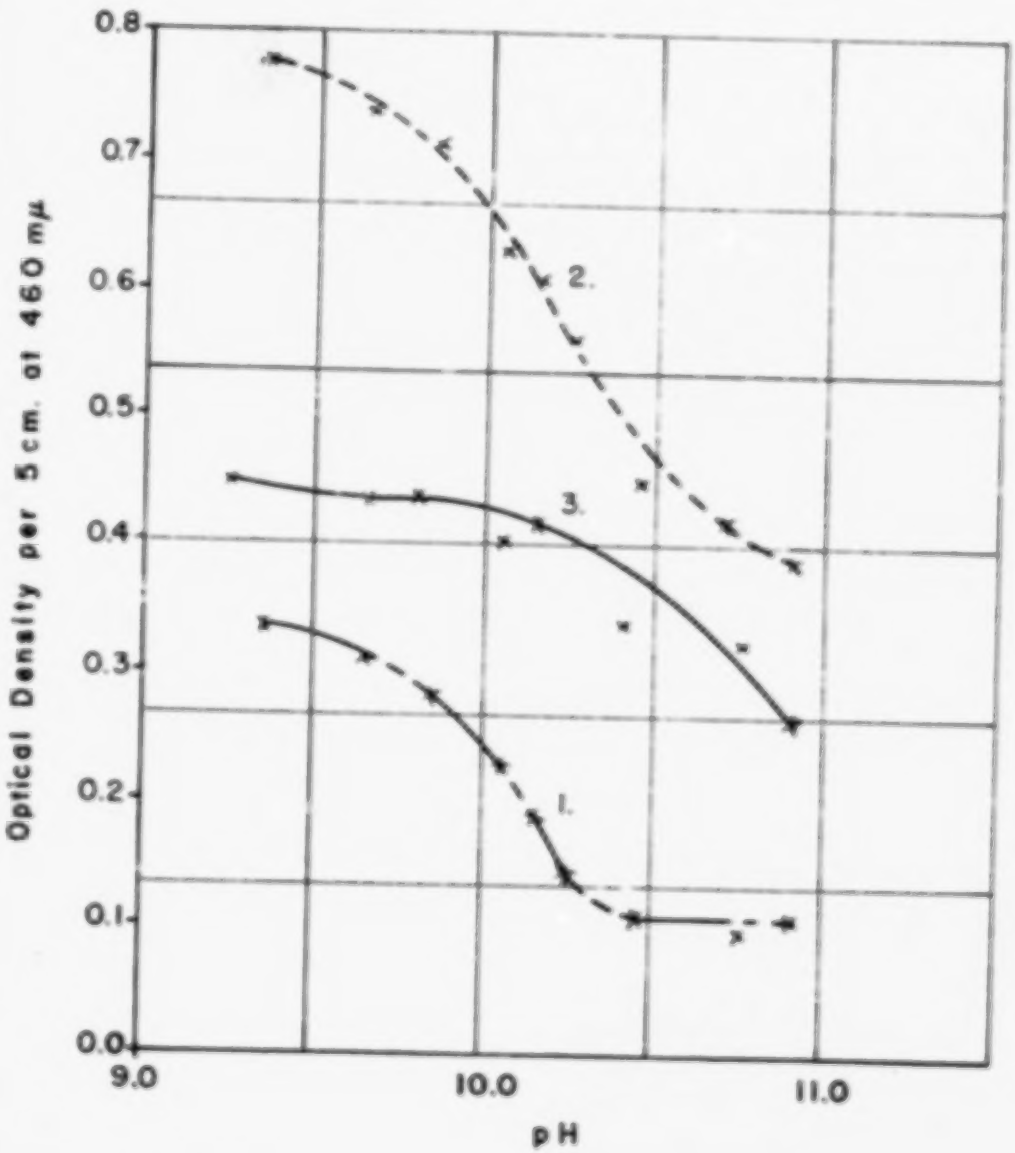

Fig. 3

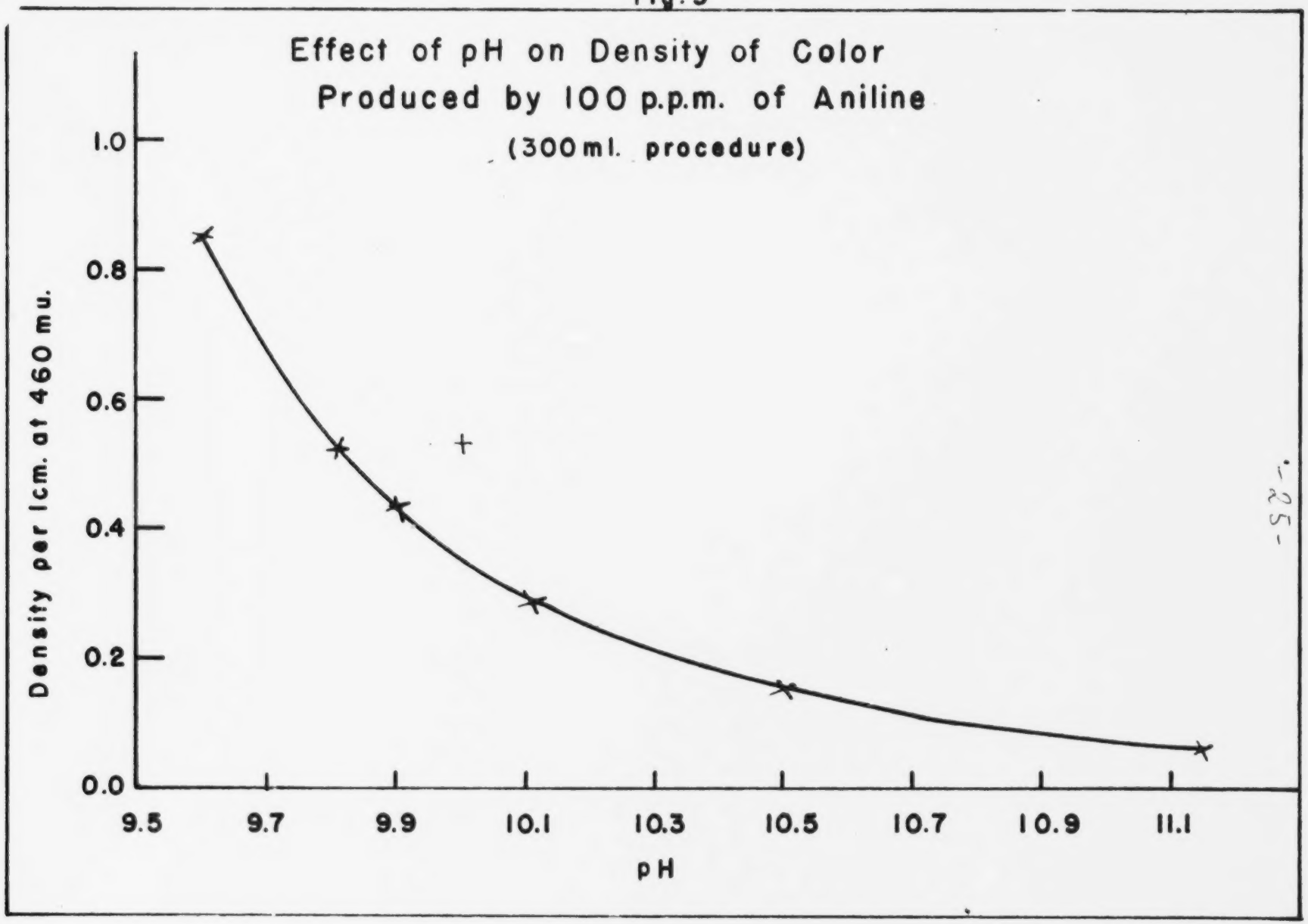
Effect of pH on Density of Color
Produced by 100 p.p.m. of Aniline
(300 ml. procedure)
Density per 1cm. at 460 mu.
1.0
0.8
0.6
0.4
0.2
0.0
9.5
9.7
9.9
10.1
10.3
10.5
10.7
10.9
11.1
pH

Fig. 4

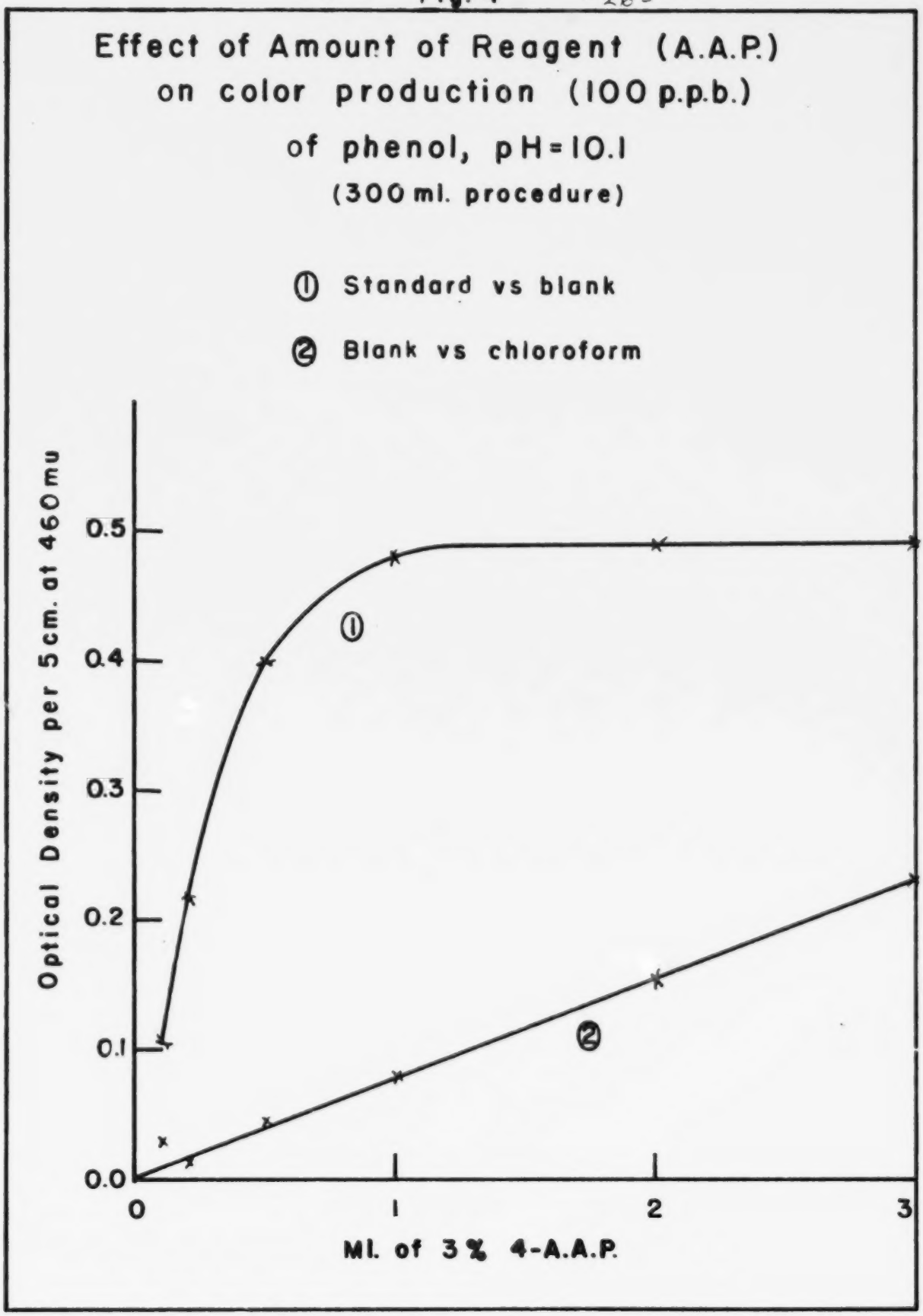
Effect of Amount of Reagent (A.A.P.)
on color production (100 p.p.b.)
of phenol, pH=10.1
(300 ml. procedure)
① Standard vs blank
② Blank vs chloroform
Optical Density per 5cm. at 460mu
0.0
0.1
0.2
0.3
0.4
0.5
0
1
2
3
Ml. of 3% 4-A.A.P.

Fig. 5

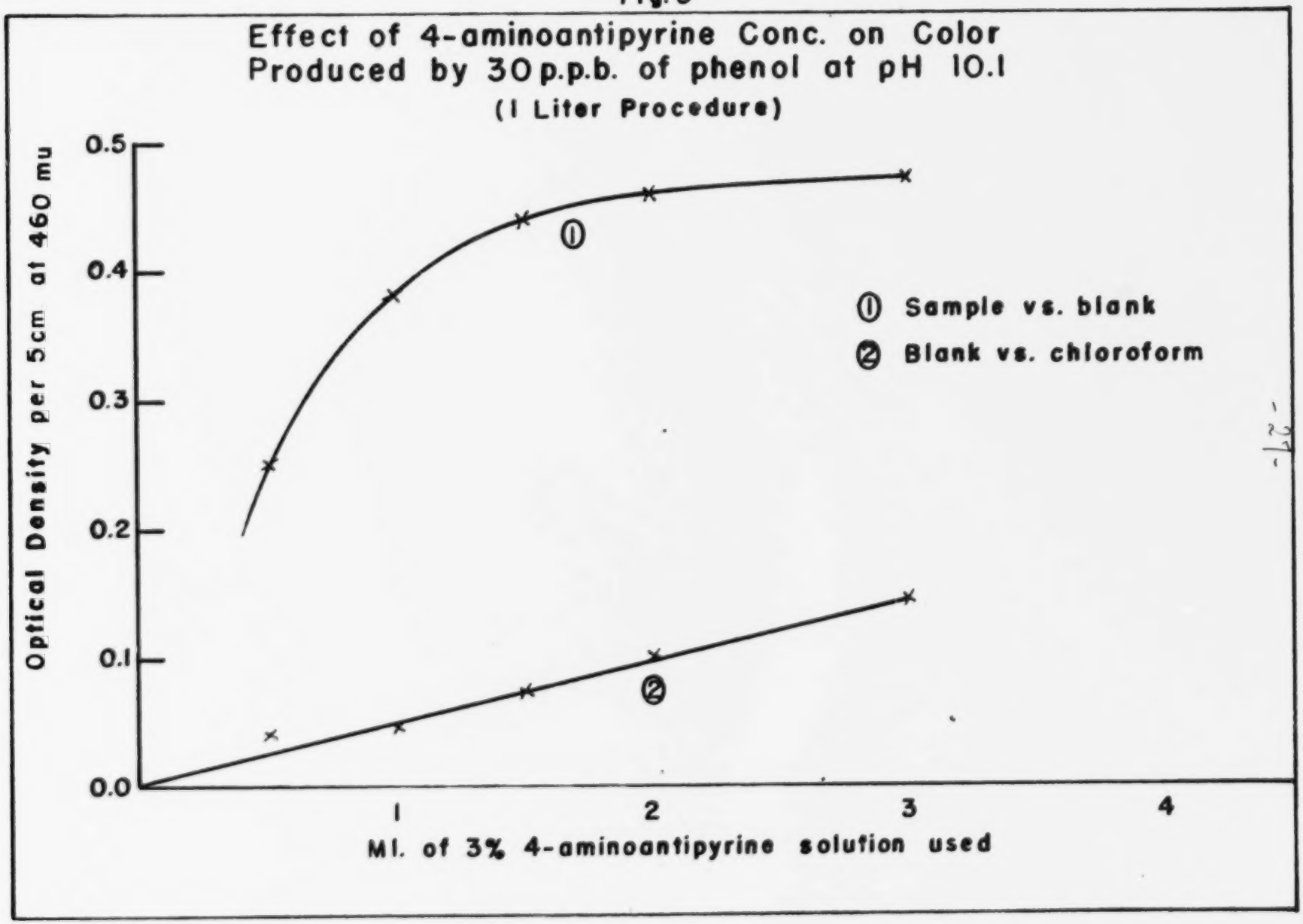
Effect of 4-aminoantipyrine Conc. on Color
Produced by 30 p.p.b. of phenol at pH 10.1
(1 Liter Procedure)
Optical Density per 5cm at 460 mu
0.5
0.4
0.3
0.2
0.1
0.0
1
2
3
4
Ml. of 3% 4-aminoantipyrine solution used
① Sample vs. blank
② Blank vs. chloroform

Fig. 6

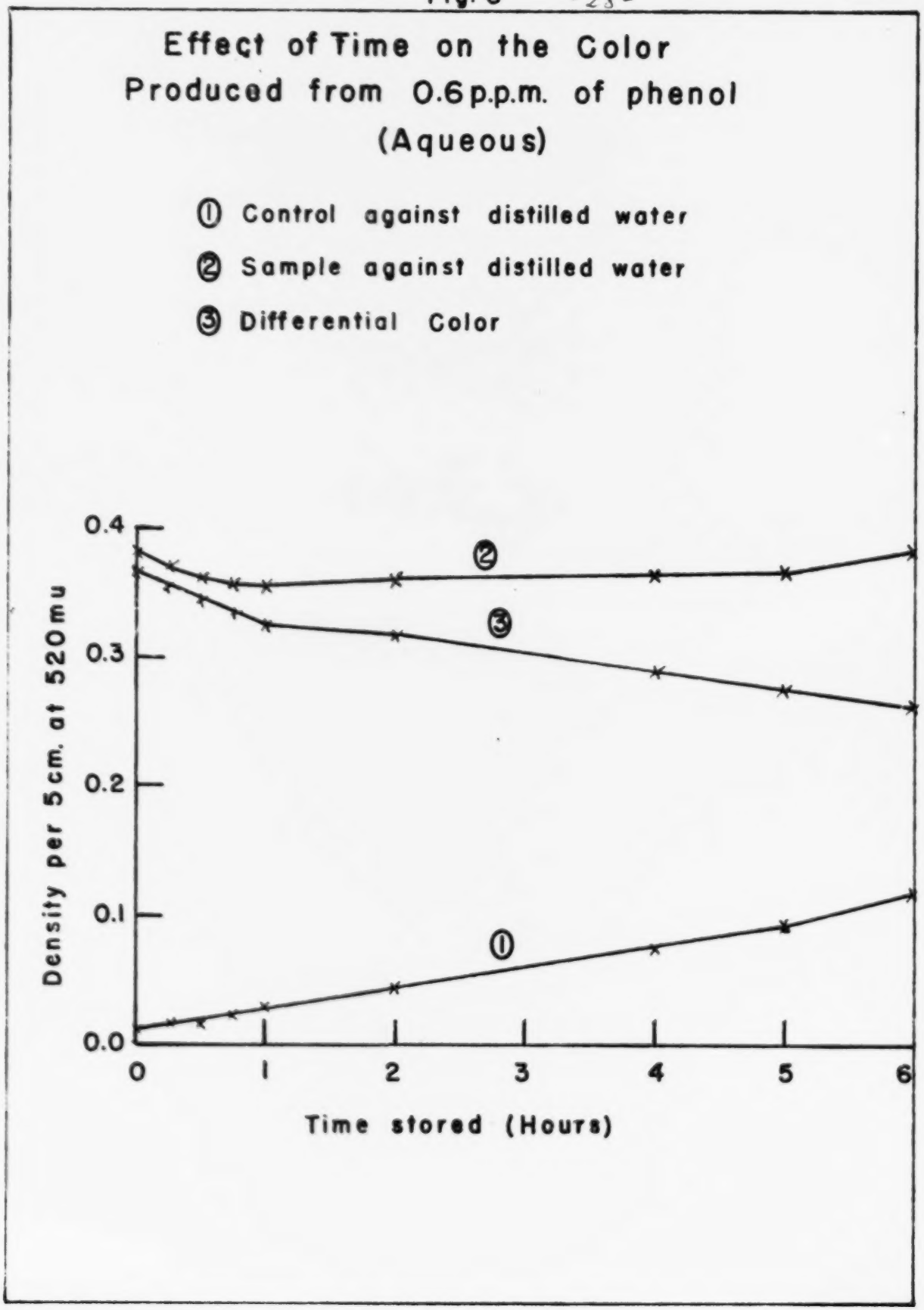
Effect of Time on the Color
Produced from 0.6p.p.m. of phenol
(Aqueous)
① Control against distilled water
② Sample against distilled water
③ Differential Color
Density per 5cm. at 520mu
0.4
0.3
0.2
0.1
0.0
0
1
2
3
4
5
6
Time stored (Hours)

FIGURE 7

COLOR PRODUCTION AS A FUNCTION OF THE CONCENTRATION OF SOME PHENOLIC MATERIALS (300 ml EXTRACTION PROCEDURE)

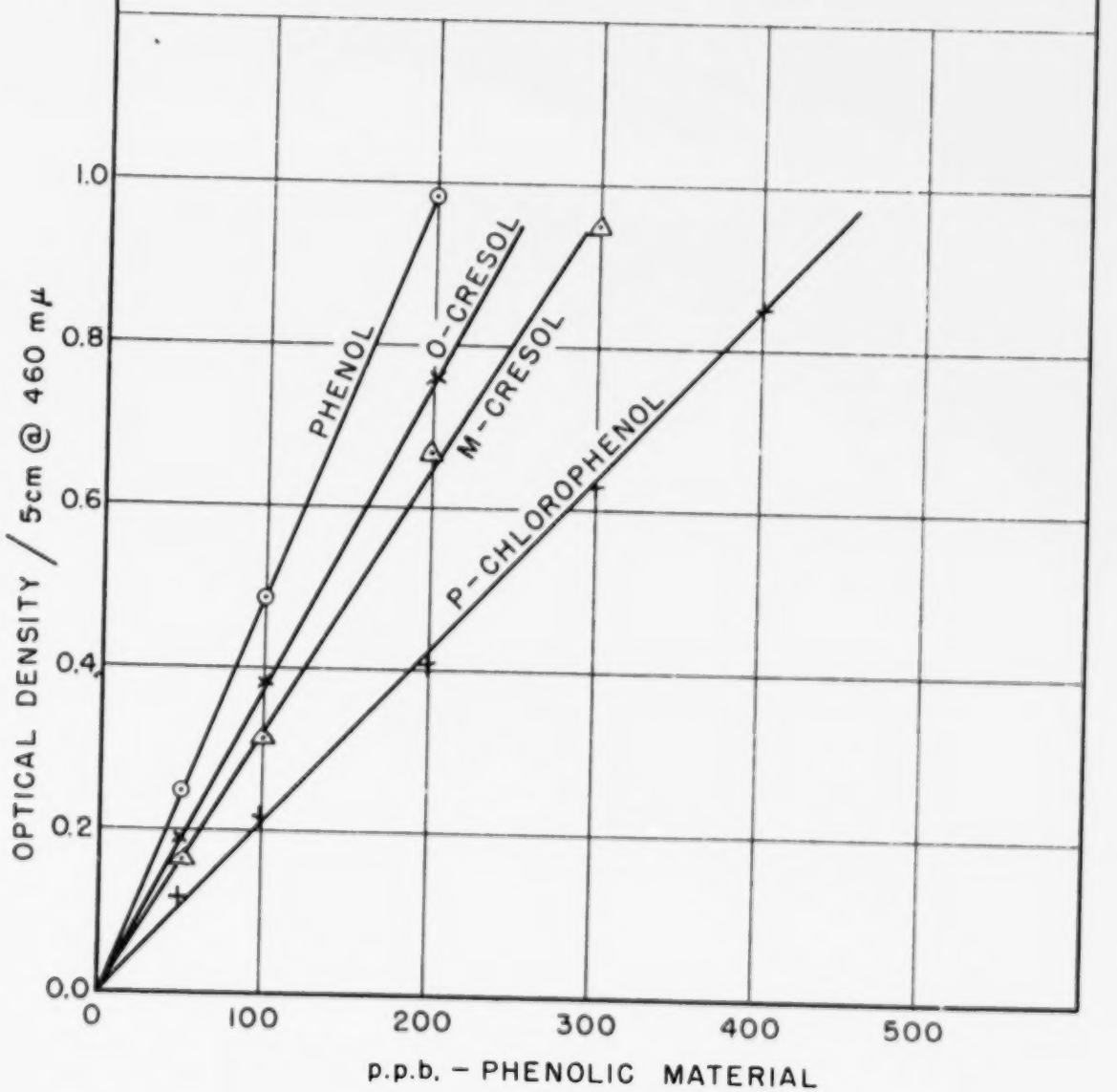

FIGURE 8

COLOR PRODUCTION AS A FUNCTION OF THE CONCENTRATION OF SOME PHENOLIC MATERIALS (1 LITER EXTRACTION PROCEDURE)

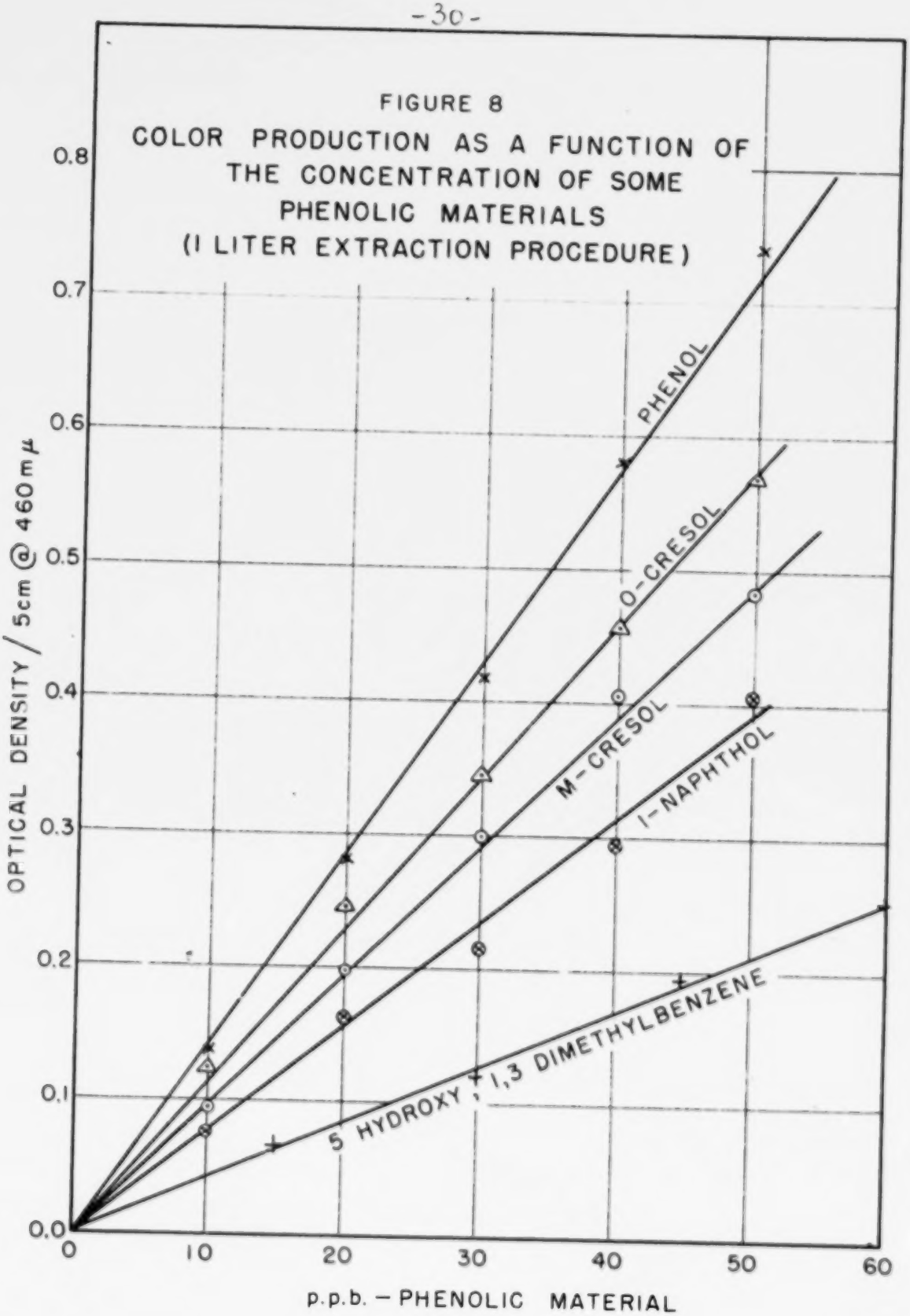

FIGURE 9

RELATIONSHIPS FOUND BETWEEN CONCENTRATION AND COLOR PRODUCED FOR PHENOL AND THE REACTIVE CRESOLS (AQUEOUS PROCEDURE)

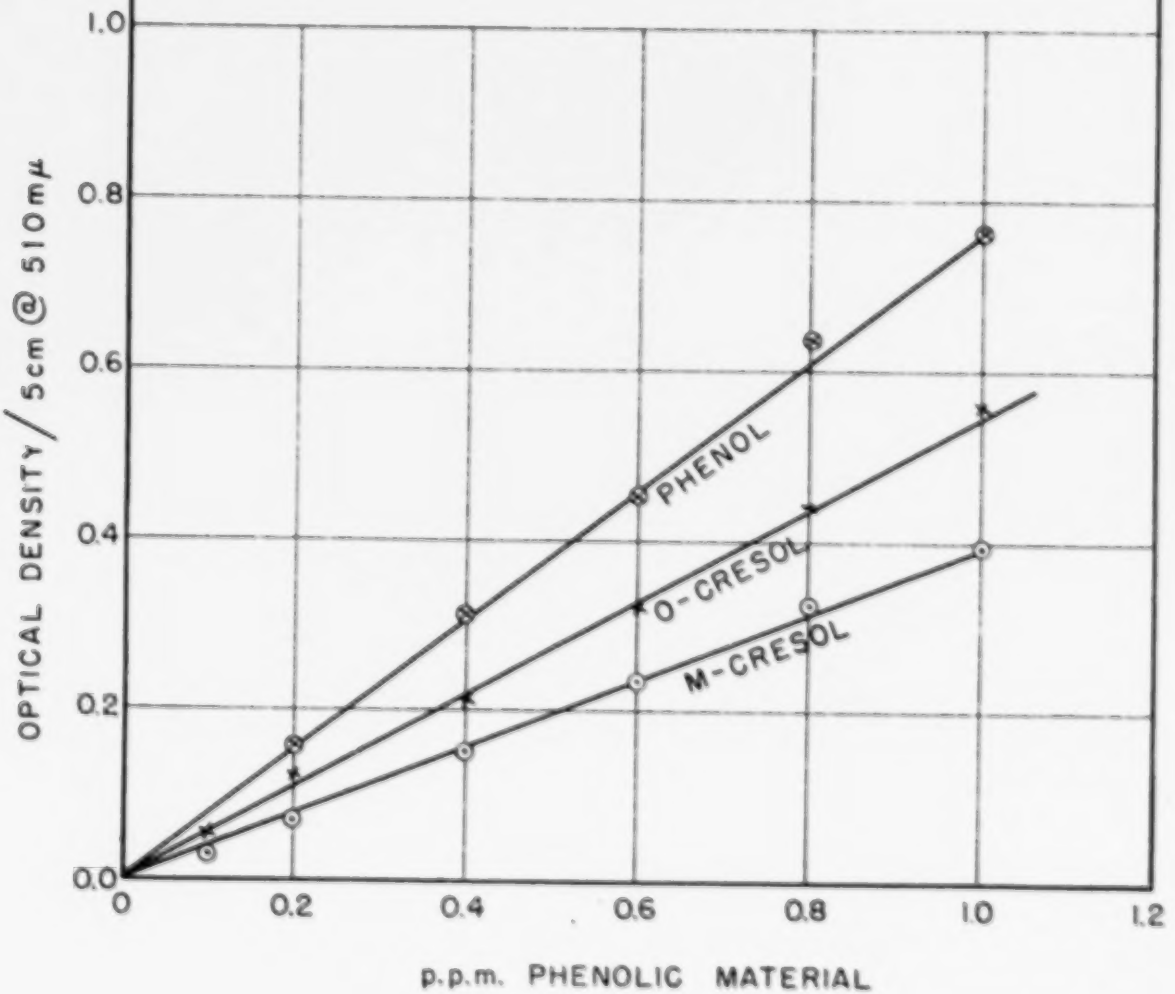

Table 1

Effect of Temperature on the Differential*
Color Produced by Varying Amounts of Phenols
(300 ml. Extraction Procedure)

p.p.b. Phenol	Optical Density/Cm. at 460 Millimicrons	
	20°C.	37°C.
50	0.055	0.071
100	0.115	0.112
200	0.225	0.233
300	0.333	0.327
400	0.442	0.430
500	0.552	0.542

* 20°C. blank versus chloroform = 0.208

37°C. blank versus chloroform = 0.280

Table 2A

Effect of Storage on Absorbancy per 5 cm. at 460 Millimicrons

(Reaction Carried out at Laboratory Temperature, Unfiltered,

Stored at Laboratory Temperature)

Time Held After Extraction (Hours)	300 ml. Procedure				1000 ml. Procedure			
			100 p.p.b. Phenol				50 p.p.b. Phenol	
	Duplicate	Blanks	Duplicates		Duplicate	Blanks	Duplicates	
0	0.191	0.199	0.709	0.682	0.138	0.134	0.880	0.910
1	0.183	0.185	0.701	0.674	0.139	0.134	0.880	0.910
2	0.186	0.188	0.708	0.678	0.140	0.135	0.902	0.945
3	0.188	0.178	0.743	0.684	0.153	0.136	0.909	0.960
4	0.187	0.179	0.700	0.671	0.140	0.135	0.900	0.918
5	0.155	0.179	0.672	0.652	0.122	0.126	0.879	0.891
24	0.146	0.166	0.700	0.670	0.115	0.148	0.907	0.902
48	0.120	0.159	0.631	0.607	0.100	0.105	0.842	0.860

Table 23

Effect of Storage Time on Optical Density at 460 Millimicrons

(Reaction Carried out at 20°C. - Filtered, Stored

at Laboratory Temperature)

Time Held After Extraction (Hours)	300 ml. Procedure				1000 ml. Procedure			
			100 p.p.b. Phenol				50 p.p.b. Phenol	
	Duplicate	Blanks	Duplicates		Duplicate	Blanks	Duplicates	
0	0.168	0.161	0.721	0.695	0.118	0.116	0.957	0.950
1	0.157	0.150	0.715	0.689	0.120	0.125	0.970	0.950
2	0.156	0.165	0.720	0.694	0.116	0.132	0.978	0.950
3	0.163	0.166	0.731	0.689	0.119	0.132	0.974	0.959
4	0.156	0.168	0.720	0.680	0.122	0.125	0.970	0.942
5	0.146	0.157	0.715	0.682	0.114	0.137	0.975	0.950
24	0.147	0.165	0.700	0.667	0.105	0.125	0.965	0.930
48	0.203	0.213	0.720	0.676	0.104	0.140	0.968	0.948
72	0.280	0.250	0.748	0.684	0.128	0.162	0.980	0.960

Table 3a

Absorption Characteristics of the Aqueous Reaction Products of Some of the Simpler Phenols.

Wave Length in Milli-microns	Per Cent of Absorbancy at 510 Millimicrons						
	Phenol	o-cresol	m-cresol	o-chloro-phenol	4-chloro-phenol	2,4-Di-chloro-phenol	2,4,6-Tri-chloro-phenol
450	51	172	95	-	-	-	95
460	52	144	92	59	66	57	92
470	72	99	89	-	-	72	86
480	86	90	93	83	87	85	91
490	95	93	98	-	94	95	99
500	99	98	100	99	99	101	98
510	100	100	100	100	100	100	100
520	97	94	96	99	97	98	98
530	90	88	90	-	-	91	89
540	80	84	81	82	81	82	80

Table 33

Absorption Characteristics of the Chloroform Extracts of the Reaction Products of Some of the Simpler Phenols

Wave Length in Milli-microns	Per Cent of Absorbancy at 460 Millimicrons						
	Phenol	o-cresol	m-cresol	o-chloro-phenol	p-chloro-phenol	2,4 Di-chloro-phenol	2,4,6-Tri-chloro-phenol
420	65	79	71	23	60	45	39
440	91	97	95	71	92	71	63
450	102	103	102	91	103	-	-
460	100	100	100	100	100	100	100
470	92	90	90	100	95	103	115
480	78	79	80	91	78	98	113
500	53	56	59	59	52	66	107
520	19	37	40	18	33	37	59

Table 4

Reaction Response Characteristics of Some Phenols

Phenolic Material	Linear Reaction	Approximate Sensitivity Limit*	Aqueous Color
Phenol	Yes	2 p.p.b.	Red
o-cresol	Yes	3 p.p.b.	Red
m-cresol	Yes	3 p.p.b.	Red
5-Hydroxy 1,3-Dimethyl Benzene	Yes	7 p.p.b.	Purple
o-chloro phenol	Yes	2 p.p.b.	Red
p-chloro phenol	Yes	4 p.p.b.	Red
2,4-Dichloro phenol	Yes	15 p.p.b.	Red
2,4,6-Trichloro phenol	Yes	50 p.p.b.	Red
2,4,5-Trichloro phenol	Yes	35 p.p.b.	Red
Pentachlorophenol	No	Over 10,000 p.p.b.	-
1-Naphthol	Yes	3 p.p.b.	Red
2-Naphthol	-	-	Green

(*) The amount in micrograms which should be detectable with reasonable certainty using the 1000 ml. extraction procedure. By running three replicates the sensitivity limit may be approximately halved.